Intracellular calcium signaling: holding the balance between health and disease.

UNIVERSITÀ DEGLI STUDI DI TORINO

EDITORIAL

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Flexibility and conservation are two hallmarks of intracellular Ca^{2+} (Ca_c) signaling. These intriguing features contributed to fix this ion as a prominent chemical regulator in cell physiology [1-5].

Calcium machinery, the entire toolkit of biomolecules generating and decoding Ca_c events inside the cell, includes a number of components, some ubiquitous and others specifically expressed by some tissues [3]. Several proteins, often clustered in multimers, compose ion channels that allow Ca^{2+} fluxes into the cytosol. They can be activated by mechanical forces, changes in membrane potential, intracellular or extracellular chemical agonists, and depletion of intracellular Ca^{2+} stores [3]. Nonetheless, some of them, typically transient receptor potential channels (TRPs), are multifunctional and can be simultaneously modulated through multiple mechanisms [6, 7].

Calcium signaling is tightly coupled to cell metabolism in a reciprocal crosstalk. Consistently, a number of diseases are linked to alterations in Ca_c signals, that can act as a cause as well as secondary effects of the pathological progression [8-11]. Furthermore Ca_c machinery, that shapes Ca_c waves and decodes Ca_c 'signature', does not work as an isolated system, being integrated with other cell signaling pathways and intracellular messengers. They include phosphoinositides ($InsP_3$, PIP_2), gasotransmitters (NO, H_2S and CO), bioactive lipids (arachidonic acid, ecosanoids, dyacilglycerol), and cyclic nucleotides (cAMP and cGMP) [5, 12-19]. cAMP is released during stimulation with hormones and growth factors. It was the first intracellular messenger discovered in 1971, and, similarly to Ca^{2+}, is universal and evolutionary conserved [20]. Recent research has revealed that cAMP-mediated signaling is highly compartmentalized and relies on a complex network of intracellular pathways, whose relative relevance depends on the specific extracellular stimulus. Mutations or genetic polymorphism involving some cAMP-related components are

associated with a variety of human diseases including long QT syndrome, cardiac dysfunctions, familial breast cancer and schizophrenia [20]. The review by A. Hofer focuses on the complex crosstalk between Ca_c- and cAMP-dependent networks. It is well known that cAMP microdomains modulate the activity of several Ca^{2+} channels, either directly or indirectly, *via* phosphorylation by cAMP-dependent protein kinase, PKA, and cAMP sensor Epac (exchange proteins activated directly by cAMP) [18]. On the other hand, the cAMP pathway is subject to modifications by Ca^{2+} and its effectors at many levels. Consistently, the authors recently reported a mechanism of cAMP release dependent on intracellular calcium store depletion, called 'Store-Operated cAMP Signaling' or 'SOcAMPS' [18-21]. Excessive activation of store-operated cAMP production has been proposed to cause human progressive polycystic liver disease [22].

Specificity of Ca^{2+}-dependent biological effects can be achieved through the selective expression and targeting of given Ca^{2+} channels and effectors. In addition, a great amount of data point to the relevance of localized intracellular events, Ca_c microdomains, due to 'signalosomes', organized clusters of Ca^{2+} channels, transporters and Ca^{2+}-dependent interactors [23, 24]. The review by Ambudkar provides a striking example of Ca_c signaling anisotropy: the exocrine function in secretory epithelia. Salivary and pancreatic gland acinar cells secrete fluid, ions, and proteins in a vectorial process that requires the coordinated regulation of ion and water channels and transporters, as well as of signaling molecules and vesicles. Spatially restricted Ca_c signals play a critical role in the regulation of this complex phenomenon [25]. The polarized co-localization of both Ca_c signaling and secretory components accounts for the physiological vectorial secretion in the exocrine salivary glands and pancreas. Pathological disruption of calcium handling is associated with autoimmune inflammatory diseases such as acute pancreatitis and Sjögren's syndrome [26, 27]. This is a fascinating example that explains how the physiopathological implications of altered Ca_c signaling at the single cell level depend on tissutal organization.

Among the major leading causes of death in the world, heart diseases are object of an impressive amount of basic and clinical studies at both cellular and tissutal levels. On the basis of its calcium-dependent excitability, it is not surprising that a number of cardiac dysfunctions in adults can be successfully related to alterations occurring in the Ca_c homeostasis of single cardiomyocytes [28, 29]. In this issue, Levi et al. investigate in detail the role of Ca_c signals in heart failure and protection from damage [30, 31]. Cardiac development, growth and differentiation are physiologically controlled by the action of several growth factors. It has been recently shown that the exogenous administration of these peptides protects the heart from failure and ischemia/reperfusion (I/R) injury [32]. Moreover, cardioprotection involves the activation of kinases, such as the so-called Reperfusion Injury Salvage Kinase (RISK) pathway, that includes phopsphoinositide 3 kinase (PI3K)/Akt and extracellular regulated signal kinases 1 and 2 (Erk1/2). Unfortunately, continuous activation of RISK cascades can result in the loss of their cardioprotective effect, hypertrophic responses and undesired secondary effects.

The authors discuss novel putative protective mechanisms that involve peptides acting through Ca_c signaling (i.e. neuregulin, urocortin and natriuretic peptides) and that, as in the case of the Neuregulin-1beta/ErbB pathway, are reaching the clinical trial relevance. Particular attention is focused on SUMOylation, a novel port-translational mechanism acting in cardioprotective processes and cardiac Ca_c handling [33].

Intracellular Ca^{2+} signals drive morphogenetic processes at tissutal level that, in turn, exert a feedback on single cells, giving rise to a functional loop between different biological scales [34, 35]. This issue includes two reviews focused on the putative multiple roles of calcium in particular biological processes, such as neuronal migration during central nervous system development and endothelial functions during the formation of new blood vessels, angiogenesis.

Lovisolo et al. discuss the role of Ca_c signaling on radial and tangential migration of neurons and neuronal precursors during the development of the nervous system [36]. Deciphering the involvement of the different Ca^{2+} influx pathways has been a major task for cellular neurobiologists, in which the main limiting factor is the limited availability of reliable and selective pharmacological tools. The authors describe the experimental strategies employed to investigate the poorly known role of Ca^{2+} channels in diseases associated

with neuronal migration. An interesting example, recently clarified, is Fetal Minamata disease (FMD), caused by exposure to methylmercury (MeHg) during development and associated with disrupted neuronal migration, maturation, and growth. MeHg inhibits granule cell migration by reducing the frequency of somal Ca^{2+} spikes through alterations in Ca^{2+}, cAMP, and insulin-like growth factor 1 (IGF1) signaling [37]. On the other hand, Lovisolo et al. investigate the putative roles of Ca^{2+}-dependent events that occur during postnatal physiological neurogenesis in the olfactory system. A general and debated question, critically analysed in this review, concerns the relative contribution of voltage-dependent and voltage-independent Ca^{2+} channels in the control of functional properties of neurosecretory cells and neuronal motility [38].

The review by Moccia et al. explores the debated involvement of endothelial progenitor cells (EPCs) in tumor neovascularisation and progression [39]. This event is suggested to integrate the classical angiogenic pathway due to sprouting from pre-existing vessels. Growing tumors may recruit EPCs from bone marrow to trigger blood vessel formation through physical engrafting into the vessel lumen and/or paracrine release of proangiogenic factors. Tumor cell-based therapy (CBT) is limited by the paucity of EPCs harvested from peripheral blood and suffers from several drawbacks [40]. A better understanding of cell signaling that controls EPC homing, proliferation and incorporation into injured tissues would help to improve this strategy. In particular, the authors investigate the potential clinical relevance of store-operated Ca^{2+} entry (SOCE), a Ca^{2+}-permeable membrane pathway that is activated upon depletion of intracellular Ca^{2+} pools. SOCE may actually contribute to the proangiogenic effects exerted by vascular endothelial growth factor (VEGF) in subpopulations of circulating EPCs.

The increasing amount of information on intracellular calcium dynamics clearly indicates that human diseases are often associated with disorders in Ca_c signaling. However, the assumption that 'correlation implies causation' should not be assumed *a priori* and warrants critical consideration. We are only beginning to integrate the plethora of experimental evidences obtained both *in vitro* and *in vivo* by the use of different models (from cell lines to animals, in healthy and altered tissues) and techniques (electrophysiology, live cell imaging). The differential structure, expression, function or targeting of Ca^{2+}-toolkit components can truly concur to the pathogenesis of some disorders, as in the case of channelopathies, in which Ca_c signals are

suitable selective targets for therapeutical approaches [11, 41-46]. Conversely, disease progression may often lead to secondary effects involving Ca_c signaling machinery. In this case, we could look at them as putative diagnostic biomarkers, mostly when they are altered in the early phases of the disease.

The main aim of this issue is to provide some critical hints on the fascinating contribution of calcium to the balance between health and disease, giving particular emphasis to the peculiarity of each biological process.

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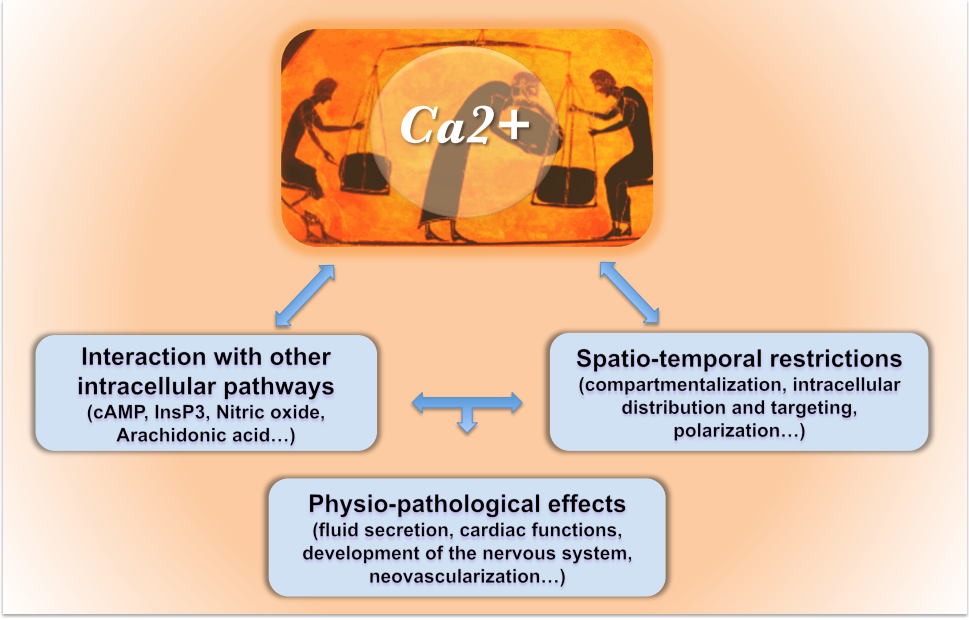

Figure legend.

Scheme showing the role of calcium in the finely tuned balance between health and disease (picture taken from ancient Knossos palace, Krete, Greece).

Complex spatio-temporal calcium dynamics, tightly and specifically integrated with other intracellular pathways, contribute to the regulation of physiology, or can concur to functional alterations, during development, postnatal and adult life. Examples in the scheme refer to those described in more detail in the present issue and are not exhaustive.